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(71) Applicant *(for all designated States except US)*: SEPRACOR, INC. [US/US]; 33 Locke Drive, Marlborough, MA 01752 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : WECHTER, William, J. [US/US]; 12876 Highview Drive, Redlands, CA 92373 (US).

(74) Agents: GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

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(54) Title: COMBINATION OF NSAIDS AND PROSTAGLANDINS AND USES THEREFOR

(57) Abstract

A combination of a non-steroidal anti-inflammatory drug and a prostaglandin of the E series is administered to individuals to treat inflammatory diseases or to reduce or prevent chronic bone demineralization or to promote bone remineralization. This drug combination is used to treat rheumatoid arthritis or other inflammatory diseases or to treat osteoporosis, bone loss associated with periodontal disease or repair of fractures. The administered amounts of the non-steroidal anti-inflammatory drug or prostaglandin are less than the typical amount of either drug that is given when that drug is individually used to achieve a desired effect. This administration feature minimizes the adverse side effects associated with these drugs.

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COMBINATION OF NSAIDS AND PROSTAGLANDINS AND USES THEREFOR

Description

Background of the Invention

Treatment of inflammatory diseases has not been satisfactory to date. Bone demineralizing states, particularly osteoporosis and loosening of metal prostheses set in bone and fractures which will not form a union, have also not been satisfactorily treated to date. A large part of the problem is the long-term nature of these, often chronic, medical problems and the lack of understanding of bone physiology associated with the homeostatic control of bone mineralization/demineralization.

The present model of bone maintenance holds that there is constant bone loss and formation and, thus, the net bone mineral balance is dynamic. Negative bone balance results from age, post-menopausal status in women, bacterially-induced inflammation at specific sites such as alveolar bone, mechanical usage or stress, metastatic disease, systemic mastocytosis and a host of other etiologies. Thus, to achieve skeletal health, a positive bone balance needs to be achieved in the spine and long bones to counteract osteoporosis, alveolar bone to counteract periodontitis, the skeleton in age or steroid-induced osteoporosis, etc. Needless to say, many endogenous agents are involved in this homeostatic system. An understanding of the influences and interaction of cytokines, prostaglandins and a variety of hormones are, at the present time, poorly realized.

Many treatments and drugs have been used to alleviate the symptoms of inflammatory diseases or bone demineralization states, sometimes leading to temporary benefit. Yet, satisfactory resolution of inflammatory diseases and the maintenance of skeletal bone remain as major unmet medical needs. The bone maintenance treatments include administration of calcitonin, calcium supplements, vitamins, vitamin D metabolites, fluoride, anabolic steroids, parathyroid hormone, diphosphonates and progestogens. Much of this work has been summarized in a Scripp Strategic Business report entitled "Recent Trends in Research and Treatment of Osteoporosis", PJB Strategic Business Reports, April, 1990. Consequently, a more effective method of treating inflammatory diseases and such bone demineralizing states, both from the standpoint of the chronic toxicity and side effects associated with the therapeutic modality as well as the efficacy, in terms of not only slowing bone loss but also leading to a positive skeletal calcium balance, would be of great value. This is particularly true at the present time when people are living longer since serious bone demineralization states are endemic to an aging population.

Summary of the Invention

The present invention is a method for the use of a combination of two classes of therapeutic agents that, until now, have not been indicated as such a combination for treating inflammatory diseases or promoting bone maintenance. This combination is administered in order to reduce or eliminate inflammatory diseases or bone

demineralization and/or promote bone remineralization. The present method and compositions are particularly useful for the treatment of osteoporosis, bone loss associated with periodontal disease, loosening of metal prostheses placed in bone, repair of non-union fractures, and other bone demineralization states. Additionally, the combinations of therapeutic agents of this invention are useful as prolonged anti-inflammatory compositions for the treatment of rheumatoid arthritis, lupus, multiple sclerosis and other inflammatory diseases.

In the method of the present invention a non-steroidal anti-inflammatory drug, or NSAID, (preferably the S-isomer when a racemic compound is involved) such as ketoprofen, flurbiprofen, indoprofen, pirprofen, indomethacin, mefanmic acid, phenylbutazone, diclofenac, naproxen, piroxicam and tolmetin is administered separately or in combination with a prostaglandin of the E series (PGE_1, PGE_2, misoprostol, 15-methyl E_1 and E_2, 16,16-dimethyl E_1 and E_2 and esters of all of the parent acids). The drugs are administered to an individual in whom inflammatory disease or bone loss is to be prevented, reduced or reversed. The NSAID is administered at a dose that inhibits the cyclooxygenase that enzymatically catalyzes the production of prostaglandins from arachidonic acid precursors in the relevant tissue. This inhibition of cyclooxygenase reduces the inflammatory effects such prostaglandin production induces. Generally, this NSAID dose is that required to induce peripheral analgesia but which avoids, most, if not all, of the complicating side effects associated with mono-

drug NSAID therapy. To this is added the minimal amount of PGE to replace the physiologic loss of PGE in the relevant tissue owing to cyclooxygenase inhibition. The dose of PGE should be sufficiently small so as to avoid substantially all of the adverse side effects associated with PGE therapy (e.g. diarrhea, abortifacient effects, etc.). When the S-isomers of the racemic aryl propionic acids are used, the dose of NSAID can be limited to less than one-half the appropriate dose of the corresponding racemic drug. Both drugs can be administered orally or topically in the buccal cavity. Alternatively, the NSAID may be administered by one of the above routes and the PGE may be administered via a transdermal delivery device.

The present compositions can be administered either singly or as a fixed combination where the amounts of the two components are, respectively, the accepted analgesic dose of the NSAID or its S-isomer and the minimal anti-ulcerogenic dose of the PGE. Typically, the NSAIDs will be given at 10 to 100% of the total daily analgesic dose and the PGE at 10 to 100% of the total daily anti-ulcerogenic dose. The ratio can be adjusted as needed to reduce observed side effects of either drug while maintaining anti-osteopenic and anti-inflammatory efficacy of the drug combination.

The present compositions and method of using them to prevent or treat inflammatory diseases or to prevent or treat bone resorptive states and to promote bone regrowth once bone resorption has occurred have many advantages over other formulations or methods of treating these conditions. For example, either drug

class used alone may possess bone sparing activity in active disease. However, the combination of these drugs acting via different biologic mechanisms are synergistic and capable of anti-inflammatory diseases activity or bone replacement beyond the effect of either an individual NSAID or PGE. When the purified S(+)-enantiomers of aryl-propionic acids are used, a dose even lower than 50% of the racemic dose is effective and useful. This can make topical or transdermal delivery of the NSAIDs practical (Cf. toothpaste or mouthwash for treatment of bone loss secondary to periodontitis).

Detailed Description of the Invention

Arylpropionic Acid (APA) and other NSAIDs have often proven beneficial in the treatment of inflammatory diseases or bone demineralizing states, particularly alveolar bone loss secondary to periodontitis (see EP 0 137 668, The Upjohn Company (Wechter), 17 July 1985; or Jeffcoat et al., "Flurbiprofen for Treatment of Periodontal Disease in Beagles", J. Periodont. Res. 21, 624-633 (1986)). It is now apparent from recent studies that an NSAID alone will not be sufficient as an effective therapy to ameliorate chronic inflammatory disease or in chronic osteoporosis secondary to menopause. While estrogen replacement therapy has been effective in slowing bone loss in this latter condition, it is not without significant side effects in many women.

The NSAIDs have also been used to treat rheumatoid arthritis and a variety of other inflammatory diseases. However, such treatment is often accompanied by a number

of side effects that limit the usefulness of the NSAID treatment. These effects are apparent in most body systems but are particularly evident in the gastrointestinal, renal and hepatic systems.

Although the dosages of the NSAIDs in the combination co-administration of the present invention are much less than in isolated NSAID therapy, the NSAIDs in the compositions of the present invention are fully effective in combating the indicated disease states and they do so without significant adverse side effects. This makes the NSAIDs of the present invention more useful for the treatment of chronic inflammatory diseases.

In the present invention, a particular combination of drugs is co-administered to reduce or eliminate inflammatory diseases or bone demineralization that accompanies such maladies as osteoporosis. Combinations of any NSAID and any PGE acid or preferably lower alkyl ester can be used to practice this invention. Preferred embodiments are selected from the following NSAIDs; the APA class (S(+) isomers being preferred over the racemic mixtures) flurbiprofen, ketoprofen, ibuprofen, naproxen, carprofen, pirprofen, fenoprofen, benoxyprofen, etc.; the salicylates including diflunisal, phenyl butazone, oxyphenbutazone and apazone; indomethacin, sulindac and their analogs; the fenamates including mefenamic acid, flurfenamic acid, meclofenamic acid and tolfenamic acid; tolmetin; oxicams, isoxicam, sudoxicam and peroxicam; as well as diclofenac, fenbufen, fenclofenac, ketorolac, etodolac and oxaprozin.

The preferred PGEs include misoprostol (particularly its active 11R, 16S isomer), PGE_1 and PGE_2 and

their lower alkyl esters, 15R-methyl PGE_1 and PGE_2 and their lower alkyl esters, 16,16-dimethyl PGE_1 and E_2, oxyprostol and their lower alkyl esters as well as other E type prostaglandins as they come into common use.

The amount of each drug that is administered in the drug combination can be significantly less than the amount given when either drug is individually administered in an attempt to achieve a specific therapeutic effect for that drug. For this reason, the adverse side effects that can accompany the administration of an NSAID (e.g., peptic ulcerations, gastrointestinal bleeding, nephritis or renal toxicity) or a prostaglandin (e.g., fever, apnea, bradycardia, tachycardia or hypotension) are minimized.

The drug combination of the invention can be administered as isolated components or as a unitary entity containing both drugs. If administered separately, the individual components can be simultaneously administered or given at different times without sacrificing efficacy. The mode of administration is a matter of choice, including parenteral, oral, topical or transdermal routes.

The pharmacological mechanism by which the invention operates is presently not known with certainty but such understanding is not essential for the operation of the invention. It is believed that the drug combination of the invention works for the following reasons.

It is known that the amelioration of inflammatory diseases or the inhibition of bone loss by administering arylpropionic acids, such as naproxen, S-flurbiprofen,

S-ibuprofen and S-ketoprofen, as well as their racemic mixtures, diminishes when these NSAIDs are chronically used to treat inflammatory diseases or osteoporosis. It appears that the absence of an ongoing inflammatory process producing PGE is the reason the inflammatory disease amelioration and the bone loss inhibition effects of administering the NSAIDs are blunted. That is, there are two pharmacological signals that are necessary for prolonged amelioration of inflammatory diseases and for bone maintenance and growth. One requirement is that a prostaglandin (PGE_1, PGE_2 and the like) be present in adequate amount to activate, respectively, specific target cells that participate in the inflammatory disease or both osteoblasts and, secondarily, osteoclasts. Such activators are ordinarily the product of the pathologic lesion (Cf. periodontitis, fracture, rheumatoid arthritis, etc.). The second signal, believed to be a hormone, factor or cytokine, is then inhibited by the NSAID resulting in amelioration of the inflammatory disease or promotion of a positive bone balance.

The problem in the case of inflammatory diseases and osteoporosis, for example, is that when there is no operating inflammatory lesion, the background level of PGE/s is critically depressed by the NSAIDs (which are also inhibitors of the cyclooxygenase enzyme requisite for prostaglandin synthesis). In the case of canine periodontal disease, the plasma levels of PGE_2 are in the range of 200-500 ng/mL. Upon successful treatment with an NSAID such as flurbiprofen, the circulating levels of PGE_2 are reduced to about 50 ng/mL. In the

absence of such an inflammatory lesion (e.g., periodontal disease) these levels are further depressed, which leads to loss of therapeutic NSAID drug effect (i.e., positive bone balance). Thus, it is necessary to supplement the PGE in those bone demineralization states (i.e., osteoporosis and open fracture) where there is not a sufficient level of PGE to activate the bone remodeling sequence via osteoblasts and osteoclasts. It is likewise necessary to supplement the PGE in heavily suppressed or quiescent periods of inflammatory disease states where the level of PGE is not maintained by immunocytes (Cf. macrophages, monocytes, etc.) that are marshalled to suppress the inflammatory disease. Thus, the disease symptoms cycle in a sinusoidal fashion as PGE concentrations oscillate.

The therapeutic treatment of this invention, therefore, consists of the chronic administration of a PG of the E series or an analog (Cf. 16,16 dimethyl-E_1 or E_2, 15R-methyl E_1 or E_2, PGE_1, or PGE_2 themselves, misoprostol (Cytotec, Searle or preferably the active 11R, 16S diasterisomer), and the like including simple esters such as in the case of misoprostol). The doses of PG are generally less than that required for gastric antisecretory activity (Cf. misoprostol less than 200 mcg given 2 to 4 times per day in man). To this treatment is added a subanti-inflammatory dose, if administered alone, of an APA (Cf. RS-flurbiprofen 10-50 mg bid, S-flurbiprofen 5-25 mg bid, RS-ketoprofen 5-25 mg qid, S-ketoprofen 2-10 mg qid, RS-ibuprofen 20-100 mg qid or S-ibuprofen 10-50 mg qid in man). Alternatively, appropriate sustained release preparations are

employed to maintain steady state blood levels of S-flurbiprofen or S-ketoprofen of about 1 mcg/mL (± 75%) or S-ibuprofen 8 mcg/mL (± 75%). The two drugs, PGE and APA, can be administered as a fixed combination or independently.

The composition of the present invention includes at least one NSAID (achiral or racemic or S-isomer of an APA) and one PGE analog employed separately as tablets, topical formulation (toothpaste or mouthwash) or transdermal preparations that will be dosed separately. Alternatively, a single oral tablet may be employed containing both drugs layered so that the NSAID is released first followed by the PGE in the innercore of the tablet. Often, the NSAIDs will be present at their analgesic dose (Cf. RS-flurbiprofen 50 mg for bid administration and the PGE at the minimal anti-ulcer dose of 200 mcg) when both drugs are given concomitantly. Typically, since these drugs act synergistically, they may be used at as little as 10-60% of the above doses. In the case of the acid NSAIDs given topically, the pH of the formulation should be maintained at acid pH (5-6). The ability of a particular formulation to have the desired effect (i.e., reduce inflammation, reduce bone loss, promote bone remineralization) can be assessed using standard techniques.

To assess the effects of the combination of therapeutic agents of this invention on the amelioration of inflammatory diseases, the adjuvant arthritis model is used since it mimics rheumatoid arthritis. In this model, arthritis is induced in Sprague-Dawley rats by injecting them with Freund's complete adjuvant or a

material that can substitute for this adjuvant in inducing arthritis. The progression of arthritis or its long-term amelioration can be followed by measuring the amount of a_1 acid glycoprotein, whose blood titer is elevated in arthritic conditions, the amount of a_2-macroglobulin plasma albumin, whose blood titer is depressed in arthritic conditions, or the size or functionality of a specified joint susceptible to arthritic changes.

Fifteen groups of 6 animals are established. To one group is administered only the adjuvant. This group serves as a positive control. To another group is administered the adjuvant and S-ketoprofen (1 mg/kg) to demonstrate that a NSAID alone has little, if any, efficacy. To still another group is administered the adjuvant and misoprostol (100 mcg/kg) to demonstrate that a PG alone has little, if any, efficacy. The remaining 12 animal groups are administered different dosages of S-ketoprofen and misoprostol where the amounts of the NSAID and PG range from quantities previously shown to be less than effective when administered alone to the quantities when either agent is solely administered. For S-ketoprofen, the dosages are either 0.1, 0.3 or 1 mg/kg. For each of these S-ketoprofen dosages, the misoprostol dosages are either 1, 10, 30 or 100 mcg/kg. With these dosages, the synergistic effect of the combination of therapeutic agents of this invention is shown. Since the NSAID and PG administration is continued for at least 30 days, the prolonged effectiveness of the protocol of administration of the therapeutic agents of this invention for ameliorating inflammatory diseases is demonstrated.

In another study, the administration of either ketoprofen, flurbiprofen, ibuprofen or naproxen is added to that of misoprostol for a panel of patients exhibiting chronic rheumatoid arthritis. This study is for an extended term and includes patients to whom subthreshold doses of the NSAID and PG are administered, compared to doses for each therapeutic agent when administered alone. This study follows a double blind protocol and uses x-ray analysis (periodic CAT scanning) of the arthritically affected joints to evaluate the effects of the administration of the combination of therapeutic agents of this invention.

A technique for assessing the reduction in bone loss or the promotion of bone remineralization for experimental animals is quantitative histomorphometry adapted from Jee et al., Bone 9, 381-389 (1988) or Li et al, Bone 10, 35-44 (1989). In this assessment, the prolonged effect of osteoporosis can be observed in the ovariectomized rat even with NSAID treatment alone for up to 60 days. Ovariectomy (OVX)-induced osteopenia is primarily due to an estrogen/ progesterone deficiency which stimulates a marked increase in bone remodeling. This leads to an imbalance of bone resorption over bone formation. The negative bone balance results in an osteopenic skeleton. This characterization of osteopenic changes in OVX rats serves as the basis for the design of the example infra which uses the OVX rat as an animal model for postmenopausal bone loss.

In this technique, after acute (10 day) or chronic treatment (up to 120 days) with a particular synergistic drug combination in an ovariectomized rat, bone status

can be assessed in comparison with contemporary control animals. Femoral bone mineral content is determined using single photon absorptometry (Norland Corporation, Fort Atkinson, WI). Three femoral sites (proximal, middle and distal) are scanned transversely. The proximal site is at the point just distal to the lesser trochanter, the middle site is at the midpoint of total femoral length, and the distal site is at the point most proximal to the femoral condyle. Bone mineral content (BMC in gm/cm) and bone width (BW in cm) are obtained, and bone mineral density (BMC/BW in gm/cm^2) calculated.

After fixation, proximal tibia, tibial shaft and metatarsals are dehydrated in graded concentrations of ethanol and defatted in acetone, then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). Frontal sections of proximal tibia and cross sections of tibial shaft and metatarsals at 230 um thickness are cut using a low-speed metallurgical saw, then ground to 100 um using a precision lapping machine (Maruto Co., Tokyo, Japan) and then microradiographed on Kodak spectroscopic plates (649-0 Eastman Kodak, Rochester, NY) at 12 kv, 25 mA for 7 minutes. Thereafter, sections are mounted on plastic slides using cyanoacrylate ester glue (Permabound 910, Adhesive, NJ) and further ground to a thickness of 20 um and coverslipped for morphometric measurements.

Microradiographs and 20 um sections are viewed qualitatively and/or quantitatively. Static and kinetic parameters are measured on 20 um sections of proximal tibia or tibial shaft for trabecular or cortical bone histomorphometry, respectively, using a digitizing image

analyzing system coupled to an epifluorescent microscope. To evaluate the changes on cancellous bone, at x156 magnification, total tissue area (T.Ar), trabecular area (Tb.Ar) and perimeter (Tb.Pm), single labeled perimeter (sL.Pm) and double labeled perimeter (dL.Pm) are measured in a 4.32 mm^2 (2.4 x 1.8 mm) area of central distal femur metaphysis located 0.6 mm proximal from growth cartilage metaphyseal junction. At x312 magnification, interlabeling distance of double labels by longitudinal bone growth (Ir.L.Wi-G) are measured. These measurements are used to calculate the following: percent trabecular area (%TB.Ar), trabecular thickness (Tb.Th), number (Tb.N), separation (Tb.Sp), percent labeled perimeter (%L.Pm), mineral opposition rate (MAR) and bone formation rates (bone area-based: BFR_1, bone surface-based: BFR_2, tissue area-based: BFR_3). For the cortical bone evaluation, total area, marrow area, cortical area, periosteal and endosteal perimeters, double and single labeled surfaces and interlabeling distances are measured on 20 um sections. Percent cortical bone, percent labeled periosteal and endosteal surfaces, periosteal and endosteal mineral apposition and formation rates are calculated according to the measurements.

Statistical differences between control and treatment groups are tested by the Kruskal-Wallis test. The data are further analyzed as a function of dose (dose dependent response) and as a function of time (time dependent response) by linear regression.

The following example is representative of the above-described technique for assessing the reduction in bone loss.

Example

Inhibition of Trabecular (Cancellous) Bone Loss in the Ovariectomized Rat by Combination Treatment with RS-Ketoprofen and Prostaglandin E_2

Thirty-one, 2-month-old Sprague-Dawley female rats were used. Twenty-four of the animals were ovariectomized (OVX) and the remaining 7 animals were sham-operated 11 days prior to the commencement of drug treatment. The animals were then divided into five treatment groups. The animals in three groups were treated with racemic ketoprofen and/or prostaglandin E_2 (PGE_2) for 10 days. The treatment groups are set forth in Table I, where n denotes the number of animals in each group:

Table I

Group No.	Treatment	n
1	Sham operation controls	7
2	OVX operation controls + vehicle	6
3	OVX + 5 mg/kg/day ketoprofen	6
4	OVX + 1 mg/kg/day PGE_2	6
5	OVX + 5 mg/kg/day ketoprofen + 1 mg/kg/day PGE_2	6

Rats in Groups 2-5 were injected subcutaneously with freshly thawed mixtures of the appropriate drug(s) and vehicle (50% ethanol/water) beginning at day 1. The volume of injection solution was 0.5 mL/kg body weight.

All rats, including the controls, received bone fluorochromes by subcutaneous injection (10 mg/kg) on day 1 (tetracycline) and 7 (calcein).

On day 11, all animals were anesthetized by intraperitoneally injecting a mixed solution of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine. The animals were euthanatized via exsanguination by cardiac puncture. One tibia was removed for histomorphometry and placed in 70% ethanol. The remaining long bones were removed and placed in vials with a small amount of saline. These samples were frozen for subsequent evaluation of mechanical strength.

Results of the treatments, as calculated from the various measurements, are summarized in Table II:

Table II

Trabecular Bone Proximal Tibia of the Rat
(Day 10)

Group	% Bone Loss (cf. Sham)	% Inhibition of OVX Bone Loss (cf. Sham)	%Trabecular Area (cf. OVX)
1	0		210*
2	47*		100
3		11	126
4		30	145
5		60	174*

* P \ .05 vs Control Comparison (cf. Sham for data in Column 2 and OVX for data in Column 4) by Student's t test. Statistical significance at this level is noted for Group 2 in Column 2 and for Groups 1 and 5 in Column 4.

It is evident from the data of Table II that only the ketoprofen and PGE_2 combination treatment (Group 5) significantly inhibited trabecular bone loss secondary to ovariectomy. Although ketoprofen or PGE_2, as an individual treatment, exhibits some inhibition of bone loss, the combination of ketoprofen and PGE_2 exhibits a previously unexpected significant inhibition of bone loss.

Visual comparisons of the proximal regions of the tibiae in each of the 5 treatment groups also provided evidence that the ketoprofen-PGE_2 combination markedly inhibits the loss of bone that accompanies ovariectomy.

Another technique for assessing the reduction in bone loss or the promotion of bone remineralization as well as the inflammation of surrounding tissue that accompanies such bone loss is the treatment of chronic destructive periodontal disease with the combination of therapeutic agents of this invention. This technique is adapted from Jeffcoat et al., J. Period. Res. 21, 624-633 (1986).

Experimental Design.

Twelve adult female beagle dogs with radiographic evidence of naturally occurring chronic destructive periodontal disease are used. The experimental design employs a 6-month pretreatment period, a 12-month treatment period, and a 6-month post-treatment period. During the 6-month pretreatment period, radiographs are taken every 3 months and a baseline, or pretreatment rate of bone loss is calculated. In addition, measurements of baseline, bone-seeking radiopharmaceutical uptake (BSRU) and gingival inflammation are performed.

At the end of the pretreatment period, the beagles are separated into two groups of dogs such that the mean rate of bone loss for the two groups is similar. Six beagle dogs are given 0.05 mg/kg (PO) of S-flurbiprofen and 30 mcg/kg (PO) of misoprostol daily in single or divided dosages. The second group of 6 dogs receives a gelatin capsule placebo daily. During the treatment and

post-treatment period, radiographs and gingival indices are taken every 3 months. BSRU is determined at 3,6 and 9 months following the start of therapy.

This experimental design allows each tooth surface to serve as its own control so that rates of bone loss, BSRU, and gingival inflammation found in the treatment period and post-treatment period can be compared to the pretreatment period.

Radiographs

Standardized radiographs of the premolar teeth are taken using modified Rinn radiographic holders. These radiographs are utilized to measure the amount and rate of alveolar bone loss.

Measurement of the alevolar bone height is performed by projecting each radiograph so that an approximately 10-fold increase in the size of the image is obtained to facilitate the reading process. The distance from alveolar crest to root apex is expressed as a percent of the tooth root length (cemento-enamel junction to root apex). All measurements are made with the assistance of a digitizer and microcomputer.

Radiopharmaceutical Uptake

At the end of the pretreatment period, and thereafter at 3-month intervals, bone seeking radiopharmaceutical uptake in the alveolar bone surrounding the second and third premolar teeth is measured. Each dog receives 1.5 mCi/kg technetium 99m-tin-diphosphonate (99mTc-Sn-MDP) intravenously. Following a 4-hour period to allow for clearance of the radiopharmaceutical from

the blood and soft tissue, uptake is measured in the alveolar bone surrounding the teeth using a miniaturized semiconductor probe radiation detector. Measurements of radiopharmaceutical uptake are taken at the alveolar crest. Uptake measurements are also taken from the nuchal crest (the bony prominence dividing the dorsal and posterior skull surfaces) before and after uptake in each quadrant is measured. The mean counts per minute of each aleveolar bone area are divided by the mean counts per minute of the nuchal crest to normalize the alveolar bone uptake data with respect to physical decay and biological clearance of the radiopharmaceutical. Repeated measurements using this methodology have been found to vary by less than 3%.

Gingival Inflammation

Gingival inflammation is assessed around each tooth using the index:

0: Clinically normal gingiva.

1: Mild inflammation--slight change in color and little change in contour.

2: Moderate inflammation--swelling obvious, glazing, redness (edematous) and chronic inflammation.

3: Severe inflammation--more swelling and redness, possible pocket formation, suppuration and tissue degeneration.

Gingival inflammation is scored by 2 examiners to minimize bias.

Statistical Analysis

Bone Loss: The rate of bone loss from one study period to the next is calculated for each tooth surface using the following formula:

$$\text{Rate} = \frac{\%\ \text{bone time 1} - \%\ \text{bone time 2}}{\text{time 1} - \text{time 2 (months)}}$$

The rate of bone loss is calculated in each of the two groups of teeth studied. The analysis of variance is used to test for statistically significant differences in the rate of bone loss over the course of the pretreatment, treatment and post-treatment period in each group. Thus, each tooth surface serves as its own control. The preceding analysis can demonstrate whether or not a treatment regimen has a significant positive or negative effect on the rate of bone loss compared to the pretreatment period.

To further evaluate the degree of effect of a regimen in comparison to a second regimen, the concept of change in rate from the pretreatment value is used. This concept is of particular importance if the rate of bone loss is increasing or decreasing in the control group. Therefore, the data are further analyzed to determine the difference between the pretreatment rate of bone loss and the rate at each time period studied. For this analysis a linear regression model is used to determine the overall rate of bone loss. Rates are calculated for: a) the overall pretreatment period, b) for the treatment period from the start of treatment to

each study interval in the treatment period, and c) from the start of the post-treatment period to each point in the post-treatment period.

Rate Change = Rate (time x)
- Rate (pretreatment period)

An unpaired t-test is used to determine if the change in rate of bone loss from the pretreatment period is significantly different in placebo versus flurbiprofen-treated group.

Bone Seeking Radiopharmaceutical Uptake: The difference between the pretreatment BSRU and the BSRU measurement at 3,6, or 9 months of the treatment periods is calculated. An unpaired t-test is used to determine if a significant difference exists between the placebo and the flurbiprofen-treated groups.

Gingival Inflammation: The mean gingival index for each group is calculated for descriptive purposes. The Mann Whitney U test is used to test for significant differences between the placebo and the flurbiprofen-treated groups.

Claims

1. Use of the combination of both a non-steroidal anti-inflammatory drug (NSAID) and a prostaglandin (PG) for the manufacture of a medicament for reducing bone demineralization or for promoting bone remineralization in an individual.

2. The use of Claim 1 wherein said NSAID is chosen from the group consisting of ketoprofen, flurbiprofen, ibuprofen, naproxen, carprofen, pirprofen, fenoprofen, benoxyprofen, diflunisal, phenylbutazone, oxyphenbutazone, apazone, indomethacin, sulindac, mefenamic acid, flurfenamic acid, meclofenamic acid, tolfenamic acid, tolmetin, oxicam, isoxicam, sudoxicam, peroxicam, diclofenac, fenbufen, fenclofenac, ketorolac, etodolac and oxaprozin.

3. The use of Claim 2 wherein said prostaglandin is chosen from the group consisting of PGE_1, PGE_2, misoprostol, 15R-methyl PGE_1, 15R-methyl PGE_2, 16,16-dimethyl PGE_1, 16,16-dimethyl PGE_2, oxyprostol, and lower alkyl esters thereof.

4. The use of Claim 3 wherein the NSAID is an arylpropionic acid.

5. The use of Claim 4 wherein the NSAID is the S isomer of said arylpropionic acid.

6. The use of Claim 4 wherein said arylpropionic acid is flurbiprofen administered to a human in amounts of about 50 mg twice a day and said prostaglandin is misoprostol administered in amounts between about 100 mcg and about 200 mcg twice a day.

7. The use of Claim 5 wherein said S isomer of said arylpropionic acid is S-ketoprofen administered to a human in amounts of about 20 mg twice a day and said prostaglandin is PGE_2 administered in amounts between about 100 mcg and about 400 mcg twice a day.

8. The use of Claim 5 wherein said combination of NSAID and PG comprises about 20 mg of S flurbiprofen and about 100 mcg of misoprostol jointly administered in unitary combination.

9. The use of Claim 5 wherein said combination of NSAID and PG comprises about 20 mg of S flurbiprofen and between about 100 mcg and 200 mcg of PGE_2 jointly administered in unitary combination.

10. The use of Claim 1 where said bone demineralization occurs as a result of a condition selected from the group consisting of osteoporosis, periodontitis, physiological stress, or anatomical stress and trauma.

11. The use of Claim 3 wherein said administration of the NSAID and PG is by a route selected from the group consisting of parenteral, oral, topical to the buccal surfaces and transdermal.

12. A composition for reducing bone demineralization in individuals comprising the combination of a non-steroidal anti-inflammatory drug (NSAID) and a prostaglandin (PG).

13. The composition of Claim 12 wherein said NSAID is chosen from the group consisting of ketoprofen, flurbiprofen, ibuprofen, naproxen, carprofen, pirprofen, fenoprofen, benoxyprofen, diflunisal, phenylbutazone, oxyphenbutazone, apazone, indomethacin, sulindac, mefenamic acid, flurfenamic acid, meclofenamic acid, tolfenamic acid, tolmetin, oxicam, isoxicam, sudoxicam, peroxicam, diclofenac, fenbufen, fenclofenac, ketorolac, etodolac and oxaprozin and said prostaglandin is chosen from the group consisting of PGE_1, PGE_2, misoprostol, 15R-methyl PGE_1, 15R-methyl PGE_2, 16,16-dimethyl PGE_1, 16,16-dimethyl PGE_2, oxypostal, and lower alkyl esters thereof.

14. The composition of Claim 13 wherein said NSAID comprises about 20 mg of S flurbiprofen and said PG comprises about 100 mcg of misoprostal jointly combined together.

15. Use of the combination of both a non-steroidal anti-inflammatory drug (NSAID) and a prostaglandin (PG) for the manufacture of a medicament for ameliorating inflammatory diseases in an individual.

16. The use of Claim 15 wherein said NSAID is chosen from the group consisting of ketoprofen, flurbiprofen, ibuprofen, naproxen, carprofen, pirprofen, fenoprofen, benoxyprofen, diflunisal, phenylbutazone, oxyphenbutazone, apazone, indomethacin, sulindac, mefenamic acid, flurfenamic acid, meclofenamic acid, tolfenamic acid, tolmetin, oxicam, isoxicam, sudoxicam, peroxicam, diclofenac, fenbufen, fenclofenac, ketorolac, etodolac and oxaprozin.

17. The use of Claim 16 wherein said prostaglandin is chosen from the group consisting of PGE_1, PGE_2, misoprostol, 15R-methyl PGE_1, 15R-methyl PGE_2, 16,16-dimethyl PGE_1, 16,16-dimethyl PGE_2, oxyprostol, and lower alkyl esters thereof.

18. The use of Claim 17 wherein the NSAID is an arylpropionic acid.

19. The use of Claim 18 wherein the NSAID is the S isomer of said arylpropionic acid.

20. The use of Claim 18 wherein said arylpropionic acid is flurbiprofen administered in amounts of about 50 mg twice a day and said prostaglandin is misoprostol administered in amounts between about 100 mcg and about 200 mcg twice a day.

21. The use of Claim 19 wherein said S isomer of said arylpropionic acid is S-ketoprofen administered in amounts of about 20 mg twice a day and said prostaglandin is PGE_2 administered in amounts between about 100 mcg and bout 400 mcg twice a day.

22. The use of Claim 19 wherein said combination of NSAID and PG comprises about 20 mg of S flurbiprofen and about 100 mcg of misoprostol jointly administered in unitary combination.

23. The use of Claim 19 wherein said combination of NSAID and PG comprises about 20 mg of S flurbiprofen and between about 100 mcg and 200 mcg of PGE_2 jointly administered in unitary combination.